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Short Communication

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First Report and the Genetic Variability of *Cucumber green mottle mosaic virus* Occurring on Bottle Gourd in Myanmar

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Abstract

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] plants showing leaf mosaic and mottle were observed in Myanmar in 2007 and shown by RT-PCR and ELISA to be infected with *Cucumber green mottle mosaic virus* (CGMMV). This is the first report of the virus occurring in Myanmar. Despite considerable differences in geographical origins, natural host species and year of sampling of 22 CGMMV isolates, we found low genetic variation of the CP gene except for isolates GR3 and GR5, which showed similarity higher than 97%; based on the MP gene, and 16 CGMMV isolates showed similarity higher than 94% in nucleotide identities by pairwise comparison. Using *Mlu*I restriction endonuclease for CP genes, the CGMMV isolates fell into three types: Type I and Type II were included in the SH group and Type III in the W group. The two CGMMV isolates from Myanmar were found to belong to Type I and Type III, respectively.

Introduction

Cucurbits are important vegetable crops in eastern and south-eastern Asia where they account for more than half of the total cucurbit crop production in the world (FAOSTAT; http://faostat.fao.org/). They are also widely cultivated in Myanmar, which is adjacent to India, and known to be the origin of many cucurbit crop species. Virus diseases cause major problems in cucurbits in Myanmar, but they have not been well studied. Elsewhere, at least 40 cucurbit-infecting viruses have been reported (Fauquet et al. 2005), of which five are tobamoviruses [*Cucumber fruit mottle mosaic virus* (CFMMV), *Cucumber green mottle mosaic virus* (CGMMV), *Kyuri green mottle mosaic virus* (KGMMV) and *Zucchini green mottle mosaic virus* (ZGMMV)]; Cucumber mottle virus (CuMoV) was recently reported in Japan as a possible new member (Kubota et al. 2006). Of these viruses, CGMMV is the most widely distributed virus and is frequently reported in many countries.

In 2007, during a survey for cucurbit viruses in Myanmar, several bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] plants had conspicuous virus disease-like symptoms. CGMMV was detected by enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) (Kim et al. 2008), in some cases in complex with a potyvirus or a putative potexvirus. This is the first report of CGMMV in Myanmar. We describe here its phylogeny and genetic variability based on coat protein (CP) nucleotide sequences and digestion with restriction enzymes.

Materials and Methods

Several bottle gourd samples with leaf mosaic and mottle symptoms were collected in Myanmar in 2007. They were tested for viruses such as *Cucumber mosaic virus* (CMV), CGMMV and KGMMV by double antibody sandwich (DAS)-ELISA (Agdia, Elkhart, IN, USA), viruses in the genus *Potyvirus* by the genus group test ELISA kit (Agdia) and viruses in the genus *Potexvirus* by RT-PCR using genus *Potexvirus* universal primer sets according to van der Vlugt and Berendsen (2002). Two isolates, designated CGMMV-Mbo1 and CGMMV-M17C, were selected for further molecular characterization. Each isolate was propagated in bottle gourd by mechanical transmission. CGMMV-KM4 and CGMMV-KM7 isolated from oriental melon (*Cucumis melo* L. var. *makuwa* Makino) in Korea (Kim et al. 2009) and CGMMV-To-TUA kindly provided by Dr T. Natsuaki, Japan were also used as reference isolates.

Total RNA was extracted from the infected leaf tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR with CKF/CKR primers was performed as described by Kim et al. (2009). The PCR fragments (1368 bp) were purified using Wizard® SV Gel and PCR Clean-Up system (Promega Corp., Madison, WI, USA) and cloned into the pGEM T-vector (Promega Corp.). At least three independent clones of each isolate were sequenced with an ABI PRISM 377 DNA Sequencer using BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

MacVector 7.2.2 software (Accelrys Software, Inc., San Diego, CA, USA) and AssemblyLIGN 1.0.9 (Accelrys Software, Inc.) were used to compare the nucleotide and deduced amino acid sequences of CGMMV isolates (Table 1). Multiple alignments were generated by Clustal W in DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/). A phylogenetic analysis of the movement protein (MP) and CP nucleotide sequences of the CGMMV isolates with KGMMV-C1 as an out-group was carried out by the neighbour-joining (NJ) algorithm calculated with 1000 bootstrap replications.

To investigate the genetic variability of CGMMV, the analysis based on nucleotide sequences of the CP region by restriction sites was performed using CLC Free Workbench 4.5 (http://www.clcbio.com/index.php?id=206). The restriction sites by 1370 enzymes from the REBASE restriction enzyme database at http://rebase.neb.com are shown on the sequence with an indication of the cut site and recognition sequence.

Table 1
CGMMV isolates and KGMMV-C1 as an out-group used for phylogenetic and restriction site analysis in this study

Virus	Strain	Original host	Country	Accession No.
CGMMV	LHP**	Cucurbits seed	China	DQ997778
	GX-G**	Cucumber	China	DQ647384
	Liaoning	Watermelon	China	EF611826
	**		China	DQ217778
	**		France	AJ429090
	GGR*	Watermelon	Greece	AY584530
	WGR*	Watermelon	Greece	AY584528
	CGR*	Watermelon	Greece	AY584529
	GR5**	Watermelon	Greece	AJ459422
	GR7**	Watermelon	Greece	AJ459423
	**	Bottle gourd	India	AY309021
	AL1**	Bottle gourd	India	AJ748352
	*	Bottle gourd	India	DQ767633
	**	Bottle gourd	India	DQ767636
	**	Zucchini	Indonesia	AB194531
	SH	Watermelon	Japan	D12505
	W	Watermelon	Japan	AB015146
	GR3**	Watermelon seed	Japan	AJ459421
	*	Watermelon	Japan	J04322
	To-TUA		Japan	AB462480
	KW	Watermelon	Korea	AF417242
	KOM	Oriental melon	Korea	AF417243
	Y*	Watermelon	Korea	AJ243353
	Y**	Watermelon	Korea	AJ245440
	**	Watermelon	Korea	AF225984
	KM4	Oriental melon	Korea	AB447984
	KM7	Oriental melon	Korea	AB447985
	M17C	Bottle gourd	Myanmar	AB510355
	Mbo1	Bottle gourd	Myanmar	AB510356
	Pak**	Bottle gourd	Pakistan	AB127937
KGMMV	C1	Cucumber	Japan	AJ295948

*Used for MP or **for CP analysis only.

Results and Discussion

The field survey revealed a high incidence of leaf mosaic and mottle symptoms in bottle gourd plants in Myanmar in 2007. CGMMV was detected in two of six collected samples and virus(es) in the genus *Potyvirus* were detected in three samples, but CMV and KGMMV were not detected. Samples reacting positively in ELISA with genus-specific antibodies to potyviruses were shown to be infected with *Papaya ringspot virus* (PRSV) (data not shown). In addition, one sample was infected by a complex of CGMMV-M17C and a putative potexvirus (Kim et al. 2008).

The nucleotide sequences coding MP and CP genes of CGMMV-Mbo1, CGMMV-M17C and the reference isolates were constructed with 795 nt and 486 nt, respectively. The identity range of the MP gene of CGMMV-Mbo1 ranged from 98% with Japanese watermelon isolate (J04322) to 93% with a Greek watermelon isolate (AY584529); that of CGMMV-M17C ranged from 99% with Indian bottle gourd isolate (DQ767633) to 94% with AY584529. Based on CP genes, the identity of CGMMV-Mbo1 ranged from 99% to 91% with the French (AJ429090) and the Greek watermelon (AJ459422) isolates, respectively. In the case of CGMMV-M17C, we also found the same range (99% to 91%) with Indian bottle gourd isolate (AY309021) and AJ459422, respectively. Comparing the sequence alignment between CGMMV-Mbo1 and CGMMV-M17C, 26 nucleotide substitutions were found on the MP and CP genes in which 25 were transitions (showing the higher incidence of C $\rightarrow$ T transitions and *vice versa*) and 1 was a transversion with no amino acid substitution.

Phylogenetic trees based on the MP or CP nucleotide sequence alignments indicated that all the CGMMV isolates are closely related but formed two distinctive groups. The two Myanmar isolates were shown to belong to separate groups with CGMMV designated W and SH with CGMMV-W and CGMMV-SH as representative isolates, respectively, CGMMV-Mbo1 being in the W group and CGMMV-M17C in the SH group (Fig. 1a,b).

Single-strand conformation polymorphism (SSCP) and/or restriction fragment length polymorphism (RFLP) are often used to determine genetic variability. Molecular variability of CGMMV isolates from watermelons and cucumbers in Korea was confirmed by SSCP and RFLP (Yoon et al. 2008). When 24 CGMMV isolates including the Myanmar CGMMV isolates were screened by digestion of genomic DNA with restriction endonucleases and, by *Mlu*I digestion, they fell into three types designated Type I, II and III (Fig. 1b,c). Most CGMMV isolates in the SH group were digested by *Mlu*I at two positions, 6031 nt and 6113 nt (6424 nt full length), and classified as Type I.

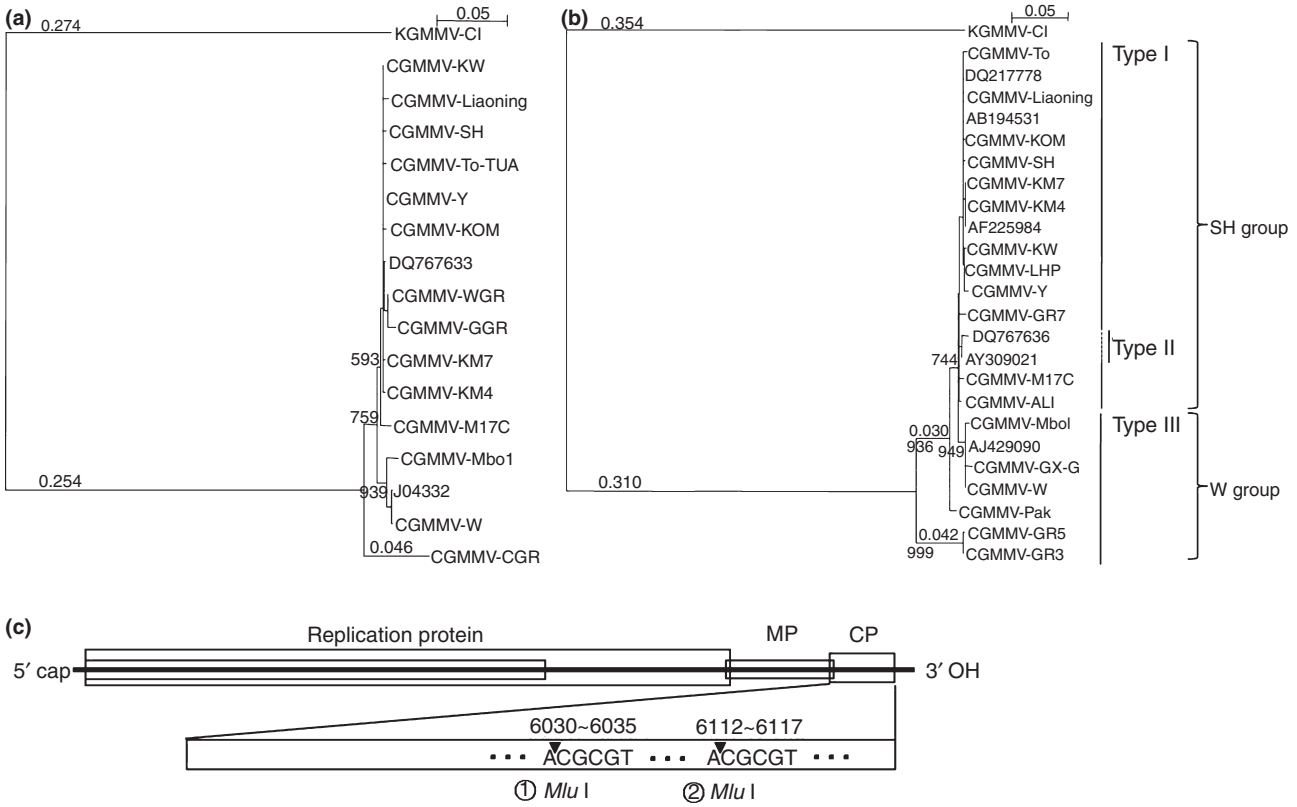

Fig. 1 Phylogenetic tree analyses of CGMMV isolates and KGMMV-C1 based on nucleotides sequences of the Movement Protein gene (a) and Coat Protein gene (b). Multiple sequence alignments were generated by using the Clustal W in DNA Data Bank of Japan homepage, and phylogenetic trees were constructed by the neighbour-joining algorithm calculated with 1000 bootstrap replications. The tree is drawn to a scale of 0.05 nt substitutions per site and bootstrap values are shown next to main relevant nodes. (c) Schematic representation of the genomic organization of CGMMV. CGMMV isolates based on the CP gene could be classified into three types by *Mlu*I

All CGMMV isolates including CGMMV-Mbo1 in the W group corresponded to Type III taking one digestion position (6031 nt). However, two isolates (DQ767636 and AY309021) from Indian bottle gourd belonging to the SH group had one digestion position at 6113 nt (Type II) that differentiated it from Type I.

We suggest the CGMMV isolates vary genetically as shown by the restriction sites, although they do not express any conspicuous genetic diversity. Generally, RNA viruses have a potential of high genetic variation associated with the error-prone replication, large populations and rapid replication (Holland et al. 1982). Nevertheless, the genetic stability of tobamovirus populations was reported as showing little variation, such as *Pepper mild mottle virus* (PMMoV) (Rodríguez-Cerezo et al. 1989) and *Tobacco mild green mosaic virus* (TMGMV) (Rodríguez-Cerezo et al. 1991; Fraile et al. 1996). This probably indicates that some factors such as negative selection and bottleneck episodes can result in low genetic diversity. We also consider that commercial exchanges of CGMMV-infected cucurbit plants, in particular virus contaminated seeds, may be responsible for the rapid invasion and dissemination of the virus worldwide. We found no significant correlation among their host species, geographical origin and the genetic distance from which the isolates were obtained. However, our results demonstrate genetic variability in CGMMV isolates occurring worldwide as well as CGMMV in Myanmar. Focusing on such genetic variability of CGMMV, this study could be a helpful baseline for understanding genetic characterization of CGMMV isolates and developing control strategies for the breeding of virus resistant cultivars and in plant quarantine.

References

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. (2005) Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. USA and UK, Elsevier Academic Press.

Fraile A, Malpica JM, Aranda MA, Rodríguez-Cerezo E, García-Arenal F. (1996) Genetic diversity in tobacco mild green mosaic tobamovirus infecting the wild plant Nicotiana glauca. *Virology* **223**:148–155.

Holland JJ, Spindler K, Horodyski F, Grabau E, Nichol S, Vande-Pol S. (1982) Rapid evolution of RNA genomes. *Science* **215**:1577–1585.

Kim OK, Mizutani T, Khin S, Lee KW, Natsuaki KT. (2008) Detection and Identification of Cucurbit Viruses in Myanmar. Istanbul, Turkey, International Union of Microbiological Societies (IUMS), pp 182.

Kim OK, Lee KW, Natsuaki KT. (2009) Occurrence and molecular characterization of *Kyuri green mottle mosaic virus* isolated from oriental melon in Korea. *J Agric Sci, Tokyo Univ Agric* **54**:71–78.

Kubota K, Orita H, Sakai J, Okuda M, Tanaka Y, Hanada K, Imamura Y, Iwanami T. (2006) Molecular and serological characterization of a new species of cucurbit-infecting Tobamovirus (Abstract in Japanese). *Jpn J Phytopathol* **72**:279–280.

Rodríguez-Cerezo E, Moya A, García-Arenal F. (1989) Variability and evolution of the plant RNA virus pepper mild mottle virus. *J Virol* **63**:2198–2203.

Rodríguez-Cerezo E, Elena SF, Moya A, García-Arenal F. (1991) High genetic stability in natural populations of the plant RNA virus tobacco mild green mosaic virus. *J Mol Evol* **32**:328–332.

van der Vlugt RAA, Berendsen M. (2002) Development of a general potexvirus detection method. *Eur J Plant Pathol* **108**:367–371.

Yoon JY, Choi GS, Choi SK, Hong JS, Choi JK, Kim W, Lee GP, Ryu KH. (2008) Molecular and biological diversities of cucumber green mottle mosaic virus from cucurbitaceous crops in Korea. *J Phytopathol* **156**:408–412.